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## A genome-wide association study of Hodgkin Lymphoma identifies new susceptibility loci at 2p16.1 (*REL*), 8q24.21, and 10p14 (*GATA3*)

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### AUTHOR CONTRIBUTIONS

RSH designed the study and obtained financial support. RSH drafted the manuscript with contributions from PB, VE, YM and SED. YM and VE performed statistical and bioinformatic analyses; PB sample coordination and laboratory analyses; BO, AL and JV performed genotyping. AJS, AA and RC provided samples and data from a study conducted at the ICR. ER initiated ELCCS; TL and ER managed and prepared Epidemiology & Genetics Lymphoma Case-Control Study samples. RFJ designed and conducted studies contributing to the UK replication series and RFJ, LS, AL and DM prepared samples and collated data. FEVL designed the Dutch NKI study and obtained financial support; NSR and MdB, were involved in identification and inclusion of Dutch cases, study design, review board approval and clinical implementation; AB coordinated collection and preparation of NKI samples. AF, KH, AE, EPvS and KR provided samples and data from German cases and controls. AD, IMN and AvdB, collection of samples and data from cHL cases ascertained through Groningen. RH, HW, TvW and RvE, ascertainment and collection of control samples from the Netherlands. HH, MM, KR, LPR, KES, HOA, BG, DM, SHD, KMS, and ETC provided samples and data from the SCALE study in Denmark and Sweden. SHD analyzed samples and provided data from Danish cHL cases. All authors contributed to the final paper. RFJ and HJ contributed equally to the paper and should be considered to have equal positional status in the author list.

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### COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests

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## Abstract

To identify predisposition loci for classical Hodgkin Lymphoma (cHL) we conducted a genome-wide association study of 589 cHL cases and 5,199 controls with validation in 4 independent samples totaling 2,057 cases and 3,416 controls. We identified three new susceptibility loci at 2p16.1 (rs1432295, *REL*; odds ratio [OR]=1.22,  $P_{\text{combined}}=1.91 \times 10^{-8}$ ), 8q24.21 (rs2019960, *PVT1*; OR=1.33,  $P_{\text{combined}}=1.26 \times 10^{-13}$ ) and 10p14 (rs501764, *GATA3*; OR=1.25,  $P_{\text{combined}}=7.05 \times 10^{-8}$ ). Furthermore, we confirmed the role of the MHC in disease etiology by revealing a strong HLA association (rs6903608; OR=1.70,  $P_{\text{combined}}=2.84 \times 10^{-50}$ ). These data provide new insight into the pathogenesis of cHL.

Classical Hodgkin Lymphoma (cHL) is a lymph node cancer of germinal center B-cell origin, characterized by malignant Hodgkin and Reed-Sternberg (HRS) cells mixed with a dominant background population of reactive lymphocytes and other inflammatory cells<sup>1</sup>. cHL is one of the most common tumors in young adults in economically developed countries, with ~1,500 cases being diagnosed each year in the UK, and the disease accounts for ~1 in 3 of all lymphomas<sup>2,3</sup>. While Epstein-Barr virus (EBV) infection may be causally related to a proportion of cases, the etiology of EBV-negative cHL remains largely unknown<sup>4</sup>.

Evidence for inherited genetic predisposition to cHL is provided by the 3 to 9-fold increased risk of cHL in first-degree relatives of cHL patients<sup>5</sup>. In the light of a possible viral basis to cHL it is interesting that cHL was the first disease to be associated with the HLA region<sup>6</sup>. Subsequent studies have reported associations between various HLA class I and class II alleles and risk of cHL<sup>7,8</sup>; specifically an association between the HLA-A\*01 and A\*02 for EBV-positive cHL<sup>9,10</sup>. Genetic variation in HLA is, however, insufficient to account for the observed familial risk of cHL<sup>11</sup>. To date no non-HLA genetic risk factors have been identified and convincingly replicated. Genome-wide linkage studies of cHL families have failed to demonstrate an additional major gene locus for cHL<sup>12</sup>. This coupled with the very high concordance of Hodgkin Lymphoma in monozygotic compared with dizygotic twins<sup>13</sup> is consistent with a genetic model of inherited susceptibility based on the co-inheritance of multiple low-risk variants.

Predicated on this hypothesis we conducted a genome-wide association study (GWAS) of 622 UK cHL cases using Illumina 660w Quad BeadChips. Genotype frequencies were compared with publicly accessible genotype data generated by the UK Wellcome Trust Case-Control Consortium 2 (WTCCC2) study of 2,930 individuals from the 1958 British Birth Cohort (58C)<sup>14</sup> and 2,737 individuals from the UK Blood Service collections (UKBS), that had been genotyped using Illumina Human 1.2M-Duo Custom\_v1 Array BeadChips (Online Methods). There was no evidence of systematic bias between these two series (Online Methods; Supplementary Figure 1), which were combined to provide genotype data on 5,667 controls. Data on 521,834 autosomal SNPs common to cases and controls were included in this analysis. After stringent quality control filtering (Online Methods; Supplementary Table 1), we analyzed 504,374 SNPs in 589 cHL cases and 5,199 controls. Principal component analysis (PCA) demonstrated that these cases and controls were genetically well matched (Supplementary Figure 2). We therefore assessed the association between each SNP and cHL risk using the Cochran-Armitage trend test without PCA adjustment. The quantile-quantile (Q-Q) plots of the negative logarithm of genome-wide *P*-values showed a strong deviation from the null distribution (Supplementary Figure 1), which could be ascribed to the strong association observed within the MHC region. After excluding 1,700 SNPs mapping to the major histocompatibility (MHC) region (6p21: 28-33Mb) there was only minimal inflation of test statistics, except at the upper tail of the distribution ( $P < 10^{-4}$ ), thereby rendering cryptic population substructure or differential genotype calling between cases and controls unlikely (genomic control inflation factor<sup>15</sup>,  $\lambda_{gc} = 1.04$ ; Supplementary Figure 1). Using principal components analyses as implemented in Eigenstrat<sup>16</sup>, correction for possible population substructure had no influence on findings for subsequently validated loci (Table 1). Furthermore, evidence for loci influencing cHL risk was provided by independent comparison with both 58C and UKBS control series (Supplementary Table 2).

This GWAS revealed multiple associations at chromosome 6, as well as suggestive associations on chromosomes 2, 5, 7, 8, 9, 10, 11 and 19 (Figure 1). To validate these associations we genotyped the HLA class II SNP rs6903608 and 10 SNPs from other regions showing an association, in the UK replication series (524 cases, 1,533 controls) (Online Methods, Supplementary Table 1). In the combined analysis, associations for 6 of

the SNPs were significant at  $P_{\text{combined}} < 1.0 \times 10^{-4}$  (Supplementary Table 3). These 6 SNPs were successfully genotyped in 3 independent case-control replication series (Online Methods, Supplementary Table 1) - SCALE (482 cases, 590 controls), Germany (498 cases, 655 controls) and Netherlands (553 cases, 638 controls). Combined analysis of all case-control series revealed genome-wide associations (*i.e.*,  $P < 5.0 \times 10^{-7}$ )<sup>17</sup> at 2p16.1, 6p21, 8q24.21 and 10p14 (Table 1; Supplementary Table 3).

In our GWAS, 42 SNPs mapping to the 4.8Mb interval at 6p21, bordered by the *TRIM27* and *MLN* genes (rs209130, 28,975,779bps and rs1547668, 33,883,424bps respectively) defining the classical MHC region, showed evidence of an association with cHL risk at  $P < 5.0 \times 10^{-7}$  (Supplementary Figure 3). The most significant associations were with SNPs mapping to HLA class II; the strongest signal was attained at rs6903608 centromeric to *HLA-DRA* ( $P = 8.12 \times 10^{-21}$ , 32,536,263bps; Supplementary Figure 3). The association between rs6903608 was consistently seen in each of the replication series,  $P_{\text{combined}} = 2.84 \times 10^{-50}$  (Table 1, Supplementary Table 3).

The association with rs1432295 ( $P_{\text{combined}} = 1.91 \times 10^{-8}$ , OR=1.22) on 2p16.1 (60,920,170bps) straddles a recombination hotspot between 2 regions of high linkage disequilibrium (LD) (Figure 2; Supplementary Figure 4). The 137Kb region defined by these two LD blocks encompasses the putative transcript *FLJ16341* and *REL* (avian reticuloendotheliosis viral oncogene homolog). *REL* encodes c-Rel, a member of the Rel/NF $\kappa$ B family of transcription factors. Constitutive activity of NF $\kappa$ B transcription factors is a hallmark of cHL<sup>1</sup> and inactivating somatic mutations of the NF $\kappa$ B signaling inhibitors play a major role in cHL pathogenesis<sup>18-20</sup>. Furthermore, studies have shown genomic amplifications of *REL* associated with increased c-Rel expression in cHL<sup>21-23</sup>.

We identified 2 SNPs on 8q24.21 associated with cHL risk, rs2019960 ( $P_{\text{combined}} = 1.26 \times 10^{-13}$ , OR=1.33) and rs2608053 ( $P_{\text{combined}} = 1.16 \times 10^{-7}$ , OR=1.20). rs2608053 mapping at 129,145,014bps localizes to a 56Kb region of LD that encompasses intron 6 of *PVT1* (Figure 2, Supplementary Figure 4). rs2019960 mapping at 129,261,453bps localizes to a 82Kb region of LD telomeric to *PVT1* (Figure 2, Supplementary Figure 4). The effects of rs2019960 and rs2608053 on cHL risk are maintained when adjusted for each other by logistic regression (OR=1.33, 95% CI: 1.23-1.44,  $P = 1.97 \times 10^{-13}$ ; and OR=1.20, 95% CI: 1.12-1.28,  $P = 1.37 \times 10^{-7}$ , respectively). Furthermore, correlation between rs2019960 and rs2608053 is poor ( $r^2 = 0.0$ ,  $D' = 0.01$  in HapMap CEU samples,  $r^2 = 0.0$ ,  $D' = 0.03$  in our control data) and comparison of haplotype frequencies provided evidence of two haplotypes differing in frequency between cases and controls (Supplementary Table 4). Because rs2019960 or rs2608053 alone cannot fully account for the association between 8q24.21 and cHL, it is possible that a unique variant in LD with and capturing the effects of both SNPs may exist. We did not, however, identify a more significant association in LD with both SNPs through imputation, making it plausible that two independent signals exist at 8q24.21.

*PVT1* is frequently involved in translocations occurring in variant Burkitt's lymphoma and murine plasmacytomas<sup>24</sup>. The *PVT1* locus encodes several microRNAs thought to be as important as *MYC* in T-lymphomagenesis and T-cell activation<sup>25</sup>. Co-activation of c-Myc

and *PVT1* has been shown in a variety of human and animal tumors<sup>26-28</sup>. The 128-130Mb genomic interval at 8q24.21 harbors multiple independent loci with different tumor specificities, including chronic lymphocytic leukemia (rs2456449; 128,262,163bps)<sup>29</sup>, prostate (rs16901979; 128,194,098bps)<sup>30</sup>, breast (rs13281615; 128,424,800bps)<sup>31</sup>, colorectal and prostate (rs6983267; 128,482,487bps)<sup>32,33</sup>, prostate (rs1447295; 128,554,220bps)<sup>34</sup> and bladder (rs9642880; 128,787,250bps)<sup>35</sup> cancer. The LD blocks defining these loci are distinct from the 8q24.21 cHL association signal ( $r^2 < 0.03$ ; Supplementary Table 5). The colorectal cancer SNP rs6983267 shows differential binding of TCF4 to an enhancer element that physically interacts with the *MYC* promoter<sup>36,37</sup>. A similar allele-specific cis-effect either on *MYC* or through *PVT1* impacting on *MYC* expression provides an attractive mechanistic basis for the 8q24.21 association with cHL risk. If the 8q24.21 locus influences risk through differential *MYC* expression, the association is intriguing since c-Myc and Rel/NFκB are the two master transcriptional systems activated in the latency III program of EBV-immortalized B-cells, which are responsible for the phenotype, growth pattern, and biological properties of cells driven into proliferation by EBV<sup>38</sup>.

The two SNPs showing an association with cHL mapping to 10p14, rs501764 ( $P_{\text{combined}} = 7.05 \times 10^{-8}$ , OR=1.25) and rs485411 ( $P_{\text{combined}} = 1.29 \times 10^{-7}$ , OR=1.22) are in strong LD ( $r^2 = 0.71$ ,  $D' = 0.95$  in HapMap CEU samples,  $r^2 = 0.69$ ,  $D' = 1.00$  in our control data) and map to a 40Kb region of LD encompassing the transcription factor and putative tumor suppressor gene, *GATA3* (GATA binding protein 3 isoform 2) (Figure 2, Supplementary Figure 4). The expression of *GATA3* is important in hematopoietic and lymphoid-cell development, acting as a master transcription factor for differentiation of T<sub>H</sub>2 cells<sup>39</sup>. A high proportion of the reactive infiltrate in cHL tumors is composed of T<sub>H</sub>2-like cells with T<sub>reg</sub> phenotype which can influence EBV-positive cHL cell growth, depending on EBV antigenic presentation by MHC molecules<sup>40</sup>. Notably, a key characteristic of HRS cells is the production of cytokines and chemokines driven by *GATA3* expression and other T-cell transcription factors<sup>41</sup>. Evidence for a biological relationship between the 2p16.1, 8q24.21 and 10p14 loci is that members of the Rel-family have differential effects on the *MYC* promoter<sup>42</sup> and *GATA3* is a target for c-Myc<sup>43</sup>.

Elucidation of the basis of each of the associations at 2p16.1, 8q24.21 and 10p14 will require fine-mapping and functional analyses. To examine if any directly typed or imputed SNPs annotate a putative transcription factor (TF) binding/enhancer element, we conducted a bioinformatic search of each of the regions of association using Transfac Matrix Database, PReMod and EEL software. At 10p14 an imputed SNP rs369421 provides the best evidence for the association signal ( $P = 6.20 \times 10^{-7}$ ) mapping within module 011553 (Supplementary Table 6, Supplementary Figure 4). Intriguingly, this module includes binding sites for ARID5B and E2F TFs. ARID5B has been previously implicated in development of acute leukemia<sup>44</sup>, and loss of PU.1, an E2F TF, has been associated with defective immunoglobulin expression in HRS cells<sup>45</sup>.

A hallmark of cHL epidemiology is the bimodal age specific incidence and it has been argued that the disease in young adults and older adults are etiologically different; in particular there is a low prevalence of EBV in younger cHL patients<sup>46</sup>. We assessed the



relationship between cHL and EBV-status, age and sex at the 6p21, 2p16.1, 8q24.21 and 10p14 loci (defined by rs6903608, rs1432295, rs2019960, rs2608053, and rs501764 genotypes) by case-only analysis using data from SCALE, UK and Netherlands replication series (1,100 cases; Supplementary Table 7). Associations at all loci were not influenced by sex after adjustment for age and EBV-status. The rs501764 association with cHL was not related to age or EBV-status (Supplementary Table 7). The HLA class II association at 6p21 was primarily driven by EBV-negative cHL after adjustment for age and sex ( $P_{\text{adjusted}}=1.63 \times 10^{-11}$ ). Similarly, rs1432295 (2p16.1) risk alleles were significantly enriched in EBV-negative cHL ( $P_{\text{adjusted}}=0.01$ ). At 8q24.21, while rs2608053 was associated with EBV-negative cHL ( $P_{\text{adjusted}}=0.01$ ), rs2019960 showed a relationship with early-onset cHL, independent of EBV-status or sex ( $P_{\text{adjusted}}=0.002$ ) (Supplementary Table 7). These phenotypic differences provide further support for two independent cHL risk loci at 8q24.21.

To explore whether any of the associations at 2p16.1, 8q24.21 and 10p14 reflect cis-acting regulatory effects on a nearby gene we searched for genotype-expression correlations in 90 EBV-transformed lymphoblastoid cell lines using previously described data<sup>47,48</sup>. We did not find any significant relationship between SNP genotype and gene expression, after adjustment for multiple testing (Supplementary Figure 5). This does not preclude the possibility that the causal variants at these disease loci have subtle effects on expression as the dynamic range of transcripts, such as *MYC*, is small. Furthermore, it is likely that only a cumulative long-term imbalance in expression in target genes will influence cHL development and expression differences may only be relevant to a specific subpopulation of B-cells, which may not be well modelled by EBV-transformed lymphocytes.

While the HLA association with cHL is a very strong genetic effect, the identification of risk variants at 2p16.1, 8q24.21 and 10p14 implicates important roles for networks involving *MYC*, *GATA3* and the NF $\kappa$ B pathway in cHL disease etiology. In the combined dataset there was some evidence for interactions between HLA (rs6903608) and 2p16.1 (rs1432295;  $P=0.05$ ) and between 8q24.21 (rs2608053) and 10p14 (rs501764 and rs485411;  $P=0.01$ ), albeit non-significant after correction for multiple testing (Supplementary Table 8). Further studies are needed to investigate possible interactions between these susceptibility loci and their interplay with EBV infection. Finally, the modest size of our study makes it likely that further risk variants for cHL can be identified through additional studies.

## ONLINE METHODS

### Patients and samples

**Genome-wide association study**—We analyzed constitutional DNA of 622 cHL patients (International Classification of Diseases [ICD] 10 codes C81.0-3) ascertained through the Royal Marsden Hospitals NHS Trust Family History study, during 2004-2008 ( $n=104$ , 63 male; mean age of diagnosis [AOD]=38, SD=16) and an ongoing national study of cHL in females ( $n=518$ , mean AOD=23, SD=6) conducted by the Institute of Cancer Research (ICR). 146 cases had been diagnosed with breast cancer subsequent to cHL diagnosis. All cases British residents and self-reported to be of European Ancestry.

For controls we used publicly accessible data generated by the UK Wellcome Trust Case-Control Consortium 2 (WTCCC2) study on 5,667 individuals from two sources: 2,930 individuals from the British 1958 Birth Cohort (58C; also known as the National Child Development Study) which includes all births in England, Wales and Scotland<sup>14</sup>; and 2,737 UK Blood Services Controls (UKBS) aged 18-69, sex- and geographically matched to reproduce the distribution of samples within 58C.

**Replication series**—The UK-replication series comprised 524 cHL cases (ICD10 C81.0-3; 290 male, mean AOD=38, SD=16) ascertained from the Scotland and Newcastle Epidemiological Study of Hodgkin Disease (SNEHD), the Young adult Hodgkin Case-Control Study (YHCCS) and the Epidemiology & Genetics Lymphoma Case-Control Study (ELCCS; [www.elccs.info](http://www.elccs.info)). Full details of SNEHD, YHCCS and ELCCS studies provided previously<sup>49-51</sup>. Briefly, SNEHD involved ascertainment of incident cases from Scotland and Northern England during 1993-1997. YHCCS was based on newly diagnosed patients aged 16-24 from Northern England during 1991-1995. ELCCS comprised patients residing in the north of England aged 16-69, with newly diagnosed, non-HIV-related HL, during 1998-2003. UK population controls obtained from SNEHD and YHCCS (n=495, 268 male, mean age 41, SD=17) and ongoing epidemiological studies of cancer conducted at the ICR (n=1,038, 524 male, mean age 60, SD=9)<sup>52</sup>.

The Scandinavian Lymphoma Etiology (SCALE) study has been described previously<sup>53,54</sup>. Briefly, SCALE is a population-based case-control study of HL and non-Hodgkin lymphomas conducted in Denmark and Sweden during 1999-2002. The study population encompassed Danish and Swedish speaking residents aged 18-74 with no history of HIV infection, solid organ transplantation or previous hematopoietic malignancy in Denmark from June 1, 2000 to August 30, 2002, and in Sweden from October 1, 1999, to April 15, 2002. Participants recruited in a Danish regional pilot phase starting November 1, 1999, were also included, as were prevalent cases of HL diagnosed since January 1, 1999 in both countries. In total, 586 patients diagnosed with cHL according to the WHO classification in the study period and 3,187 controls representing 91% and 71% of eligible cases and controls, respectively, participated in the study, which included telephone interview and blood sampling. For the present investigation, DNA from 482 cases (82% of all SCALE cHL cases, 282 male, mean AOD=40, SD=16) and from 255 Danish controls was extracted from dried filter paper blood spots with Extract-N-AmpT as per manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA) and subjected to whole genome amplification with AmpliQ Genomic Amplifier Kit (Ampliqon, Denmark)<sup>55</sup>. In addition, germline DNA extracted from buffy coat for 335 Danish SCALE controls (randomly selected from 590 controls) was also included. (Mean age for combined SCALE controls 59, SD=13).

The Netherlands replication series comprised: (i) 281 cHL patients (149 male, mean AOD=36, SD=15) collected from the north of the Netherlands diagnosed during 1997-2000 as part of an ascertainment by the University Medical Centre Groningen; (ii) 272 cHL cases, 97 diagnosed with breast cancer subsequent to cHL (mean AOD=24, SD=6). These patients were selected in the framework of an ongoing case-control study of risk factors for breast cancer after HL conducted by the Netherlands Cancer Institute, Amsterdam, within a larger cohort study of women treated for cHL before age 60, during 1965-1995 and who survived



at least 5 years. Patient selection, methods of data/blood collection and DNA isolation described previously<sup>56-58</sup>. Samples from healthy blood donors, aged 19-69, ascertained through medical centers in Groningen (mean age=52, SD=11) and Leiden (mean age=47, SD=12), served as controls.

The German replication series comprised 498 cHL patients ascertained by the German Hodgkin Study Group during 1998-2007 (292 male, mean AOD=34, SD=12). Controls were 655 healthy blood donors from Mannheim, located 200km from Cologne (381 male, mean age=36, SD=13).

**EBV status of tumors**—EBV status of cHL tumors was determined by immunohistochemical staining for EBV latent membrane antigen (LMP)-1 and/or EBV EBER *in situ* hybridization using sections of paraffin-embedded material<sup>53,59</sup>.

**Ethics**—Collection of blood samples and clinico-pathological information from subjects was undertaken with informed consent and relevant ethical review board approval in accordance with the tenets of the Declaration of Helsinki.

**Genotyping**—DNA extracted from samples using conventional methodologies and quantified using PicoGreen (Invitrogen, Carlsbad, USA).

Genotyping of cHL cases in the GWAS conducted using Illumina Infinium HD Human660-Quad BeadChips according to manufacturer's protocols (Illumina, San Diego, USA). DNA samples with GenCall scores <0.25 at any locus considered "no-calls". A SNP was considered failed if fewer <95% of DNA samples generated a genotype at the locus. Cluster plots manually inspected for all SNPs considered for replication.

We used data on controls from the 1958 Birth Cohort (58C) and National Blood Service (UKBS) which had been generated by the WTCCC. Genotyping of both sets of controls was conducted using Illumina Human 1.2M-Duo Custom\_v1 Array chips. SNP calling performed using Illuminus Software. Full details of genotyping, SNP calling and QC reported previously ([www.wtccc.org.uk](http://www.wtccc.org.uk)). Concordant with previous findings<sup>17</sup> comparison of the two control series showed little evidence for systematic bias (inflation factor  $\lambda=1.022$ ; Supplementary Figure 1).

Validation and replication of associations were performed using competitive allele-specific PCR KASPar chemistry (KBiosciences Ltd, Hertfordshire, UK). Primers and probes used available on request. Samples having SNP call rates <90% excluded from analysis. To ensure quality of genotyping in all assays, at least two negative controls and 1-2% duplicates (showing a concordance >99.99%) were genotyped. We performed cross-platform validation and sequenced a random series of 96 samples to exclude technical artifact confirm genotyping accuracy (concordance>99.9%).

**Statistical and bioinformatic analysis**—We applied pre-determined quality-control metrics to the GWAS data. We restricted analyses to samples for whom >95% of SNPs were successfully genotyped, eliminating 12 cases. We computed identity-by-state (IBS) probabilities for all pairs to search for duplicates and closely related individuals amongst

cases and controls (defined as IBS  $\geq 0.80$ , thereby excluding first-degree relatives). For all identical pairs the sample with highest call rate was retained, eliminating 2 cases. To identify individuals with possible non-Western European ancestry, we merged our case and control data with HapMapII samples (60 western European [CEU], 60 Nigerian [YRI], 90 Japanese [JPT] and 90 Han Chinese [CHB]). For each pair of individuals we calculated genome-wide IBS distances on markers shared between HapMap and our SNP panel, used as dissimilarity measures upon which to perform principal component analysis. The first two principal components for each individual were plotted; any individual not present in the main CEU cluster (*i.e.* 5% furthest from cluster centroids) was excluded. We removed 30 cases with non-CEU ancestry (some of which had poor call rates) and 1 WTCCC2 control which was a duplicate case. We excluded SNPs with minor allele frequency  $<1\%$ , and call rate  $<95\%$  (cases or controls) and those showing departure from Hardy-Weinberg equilibrium ( $P < 10^{-5}$ ) in controls. For replication and validation analysis call rates were  $>95\%$  per 384-well plate for each SNP; cluster plots visually examined by two researchers.

Main analyses were undertaken using R(v2.6), Stata10 (State College, Texas, US) and PLINK(v1.06). Association between each SNP and cHL risk was assessed by the Cochran-Armitage trend test. The adequacy of case-control matching and possibility of differential genotyping of cases and controls were formally evaluated using quantile-quantile (Q-Q) plots of test statistics. The inflation factor  $\lambda$  was based on the 90% least significant SNPs<sup>15</sup>. We adjusted for possible population substructure using Eigenstrat software<sup>16</sup>. Odds ratios (ORs) and associated 95% confidence intervals (CIs) were calculated by unconditional logistic regression. Meta-analysis was conducted using standard methods<sup>60</sup>. Cochran's Q statistic and  $I^2$  statistic were calculated to test for heterogeneity and quantify total variation due to heterogeneity; large heterogeneity typically defined as  $I^2 \geq 75\%$ <sup>61</sup>. We conducted a pooled analysis incorporating Eigenstrat-adjusted *P*-values from the GWAS using the weighted Z-method implemented in the program METAL. We examined each SNP for dose response by comparing 1-d.f. and 2-d.f. logistic regression models, adjusting for stage using a likelihood ratio test, and examined the combined effects of multiple SNPs by evaluating the effect of adding an interaction term on the model by using a likelihood ratio test and adjusting for stage. Associations by sex, age and EBV-status were examined by logistic regression in case-only analyses.

Prediction of the untyped SNPs was carried out using IMPUTEv2, based on HapMapIII/Release27 (Feb2009, NCBI B36, dbSNP26) and 1000genomes. Imputed data were analysed using SNPTESTv2 to account for uncertainties in SNP prediction. LD-metrics between HapMap SNPs were based on HapMapIII/Release27, viewed using Haploview(v4.2) and plotted using SNAP. LD-blocks defined on the basis of HapMap recombination rate (cM/Mb) as defined using Oxford recombination hotspots<sup>62</sup> and on the basis of distribution of confidence intervals defined by Gabriel *et al.*<sup>63</sup>

To annotate potential regulatory sequences within disease loci we implemented *in silico* searches using Transfac Database(v7.29)<sup>64</sup>, PReMod10<sup>65</sup> and EEL<sup>66</sup>.

**Relationship between SNP genotypes and expression levels**—To examine for a relationship between SNP genotype and expression levels of *GATA3*, *REL*, and *MYC* in

lymphocytes we made use of publicly available expression data generated from analysis of 90 Caucasian derived Epstein-Barr virus-transformed lymphoblastoid cell lines using Sentrix Human-6 Expression BeadChips (Illumina, San Diego, USA)<sup>47,48</sup>. Online recovery of data performed using WGAViewer(v1.25). Differences in distribution of mRNA expression levels between SNP genotypes were compared using a Wilcoxon-type trend test<sup>67</sup>.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENTS

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## URLs

The R suite can be found at <http://www.r-project.org/>

Detailed information on the tag SNP panel can be found at <http://www.illumina.com/>

dbSNP: <http://www.ncbi.nlm.nih.gov/projects/SNP/>

HapMap: <http://www.hapmap.org/>

1000Genomes: <http://www.1000genomes.org/>

1958 Birth Cohort: <http://www.cls.ioe.ac.uk/studies.asp?section=000100020003>

KBioscience: <http://kbioscience.co.uk/>

WGAViewer: <http://www.genome.duke.edu/centers/pg2/downloads/wgaviewer.php>

SNAP <http://www.broadinstitute.org/mpg/snap/>

IMPUTE: <https://mathgen.stats.ox.ac.uk/impute/impute.html>

SNPTEST: <http://www.stats.ox.ac.uk/~marchini/software/gwas/snpctest.html>

EEL: <http://www.cs.helsinki.fi/research/algodan/EEL/>

PReMod: <http://genomequebec.mcgill.ca/PReMod/welcome.do>

Transfac Matrix Database: <http://www.biobase-international.com/pages/index.php?id=transfac>

JASPAR2 database: <http://jaspar.cgb.ki.se/>

EIGENSTRAT: <http://genepath.med.harvard.edu/~reich/Software.htm>

Wellcome Trust Case Control Consortium: [www.wtccc.org.uk](http://www.wtccc.org.uk)

METAL: [www.sph.umich.edu/csg/abecasis/metal](http://www.sph.umich.edu/csg/abecasis/metal)

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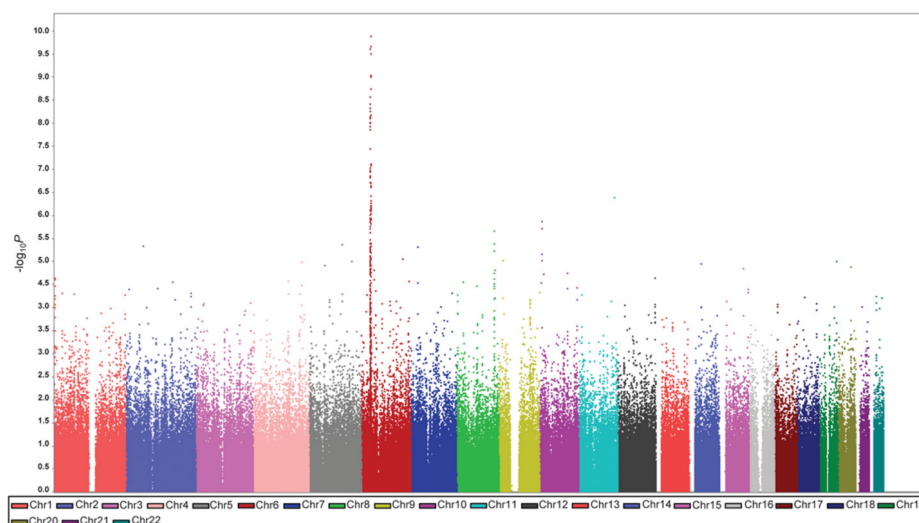
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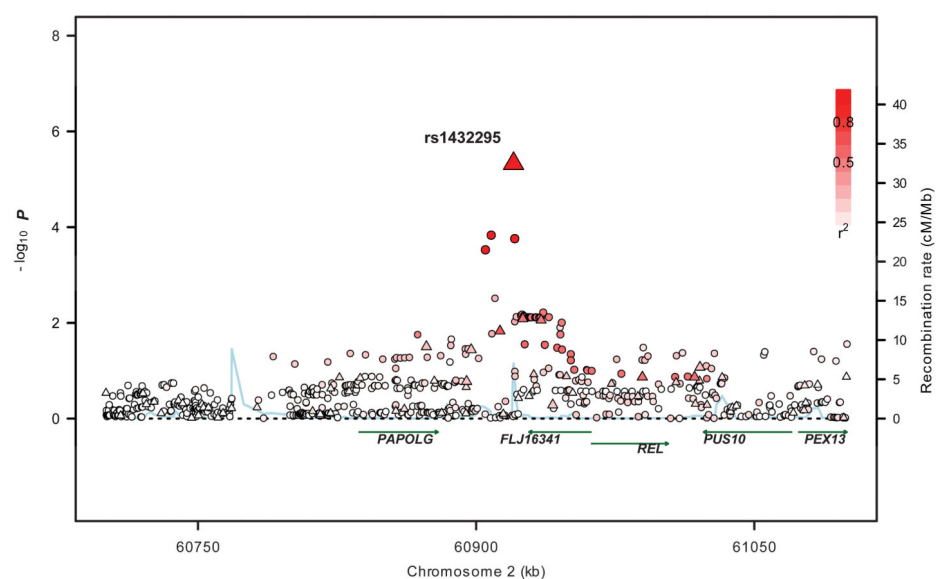
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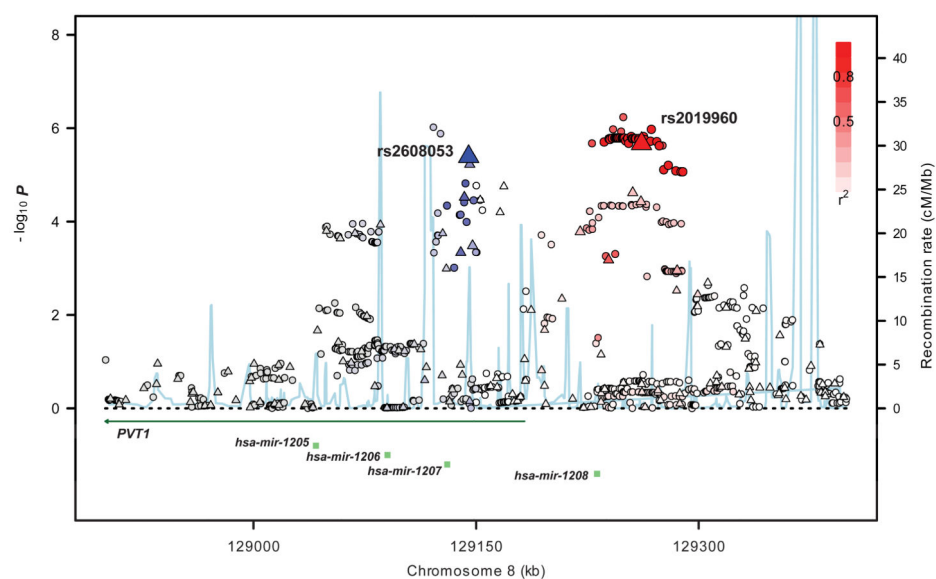
**Figure 1. Genome-wide association results from the initial GWAS**

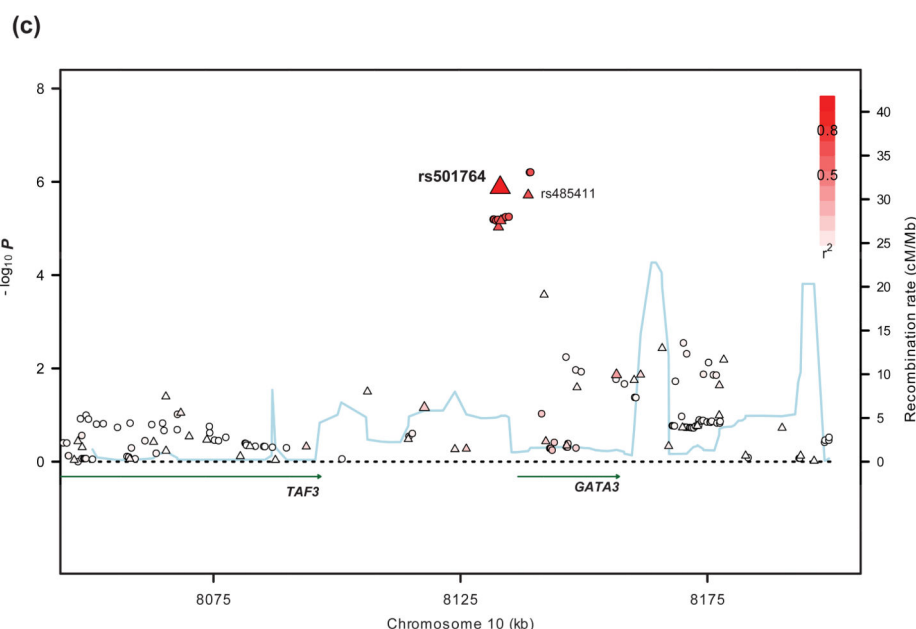
Shown are the genome-wide  $P$ -values obtained using the Cochran-Armitage trend test from 504,374 autosomal SNPs in 589 HL cases and 5,199 controls.  $P$ -values ( $-\log_{10}P$ , y axis) are plotted against their respective chromosomal positions (x axis). Each chromosome is depicted in a different color. The points with  $P < 10^{-10}$  were truncated; the smallest  $P$  value is  $8.12 \times 10^{-21}$ .

(a)



(b)





**Figure 2. Regional plots of association results and recombination rates for 2p16.1, 8q24.21, and 10p14 susceptibility loci**

(a-c) Association results of both genotyped (triangles) and imputed (circles) SNPs in the GWAS samples and recombination rates within the three loci: (a) 2p16.1; (b) 8q24.21; (c) 10p14. For each plot,  $-\log_{10} P$  values (y-axis) of the SNPs are shown according to their chromosomal positions (x-axis). The top genotyped SNP in the combined analysis is labeled by rs ID. The color intensity of each symbol reflects the extent of LD with the top genotyped SNP – red/blue ( $r^2 > 0.8$ ) through to white ( $r^2 < 0.2$ ). Genetic recombination rates (cM/Mb), estimated using HapMap CEU samples, are shown with a light blue line. Physical positions are based on build 36 (NCBI) of the human genome. Also shown are the relative positions of genes and transcripts mapping to each region of association. Genes and miRNAs have been redrawn to show the relative positions; therefore, maps are not to physical scale.

**Table 1**  
**Summary results for six SNPs associated with classical Hodgkin's lymphoma risk**

Chr	SNP	Location (bps) <sup>a</sup>	Gene <sup>b</sup>	Risk allele	RAI <sup>c</sup> control	GWAS		Replication studies		Combined			
						OR <sup>d</sup> (95% CI)	P *	OR (95% CI)	P	OR (95% CI)	P †	P <sub>het</sub>	I <sup>2</sup>
2p16.1	rs1432295	60,920,170	REL	G	0.40	1.33 (1.18-1.51)	4.69 × 10 <sup>-6</sup>	1.17 (1.08-1.27)	1.91 × 10 <sup>-4</sup>	1.22 (1.14-1.30)	1.91 × 10 <sup>-8</sup>	0.25	26%
6p21.32	rs6903608	32,536,263	HLA-DRA	G	0.27	1.81 (1.60-2.05)	8.12 × 10 <sup>-21</sup>	1.65 (1.52-1.80)	4.95 × 10 <sup>-32</sup>	1.70 (1.58-1.82)	2.84 × 10 <sup>-50</sup>	0.12	46%
8q24.21	rs2608053	129,145,014	PVT1	G	0.52	1.33 (1.18-1.50)	4.06 × 10 <sup>-6</sup>	1.15 (1.06-1.24)	8.38 × 10 <sup>-4</sup>	1.20 (1.12-1.28)	1.16 × 10 <sup>-7</sup>	0.10	48%
8q24.21	rs2019960	129,261,453	PVT1	G	0.23	1.38 (1.21-1.58)	2.14 × 10 <sup>-6</sup>	1.31 (1.19-1.44)	8.92 × 10 <sup>-9</sup>	1.33 (1.23-1.44)	1.26 × 10 <sup>-13</sup>	0.89	0%
10p14	rs501764	8,133,040	GATA3	C	0.19	1.42 (1.23-1.63)	1.33 × 10 <sup>-6</sup>	1.18 (1.07-1.30)	1.28 × 10 <sup>-3</sup>	1.25 (1.15-1.36)	7.05 × 10 <sup>-8</sup>	0.09	51%
10p14	rs485411	8,133,191	GATA3	A	0.25	1.35 (1.18-1.54)	6.83 × 10 <sup>-6</sup>	1.17 (1.07-1.28)	8.68 × 10 <sup>-4</sup>	1.22 (1.13-1.32)	1.29 × 10 <sup>-7</sup>	0.25	26%

Detailed data including genotype counts are shown in Supplementary Table 3.

<sup>a</sup>Chromosome location based on NCBI Human Genome Build 36 coordinates.

<sup>b</sup>Putative candidate genes mapping within 50 kb of respective SNPs.

<sup>c</sup>Risk allele frequency.

<sup>d</sup>Odds ratio with 95% Confidence Interval.

\* EIGENSTRAT-adjusted P-values: rs1432295, P = 8.87 × 10<sup>-6</sup>; rs6903608, P = 2.93 × 10<sup>-17</sup>; rs2608053, P = 4.20 × 10<sup>-6</sup>; rs2019960, P = 9.14 × 10<sup>-7</sup>; rs501764, P = 1.67 × 10<sup>-6</sup>; rs485411, P = 1.25 × 10<sup>-5</sup>.

† Combined P-values using adjusted data rs1432295, P = 5.02 × 10<sup>-9</sup>; rs6903608, P = 1.86 × 10<sup>-46</sup>; rs2608053, P = 1.84 × 10<sup>-8</sup>; rs2019960, P = 4.01 × 10<sup>-14</sup>; rs501764, P = 1.80 × 10<sup>-8</sup>; rs485411, P = 4.51 × 10<sup>-8</sup>.